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Analysis of protein enrichment during single- and multi-stage tribo-electrostatic bioseparation processes for dry fractionation of legume flour

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ABSTRACT

The potential of a dry fractionation approach for the production of native plant protein concentrates has been validated for navy bean (*Phaseolus vulgaris*) flour as a model system utilizing a lab-scale tribo-electrostatic separator. For this approach, protein and carbohydrate particles in pin-milled flour were tribo-charged to different levels before being fractionated according to their acquired charge under the influence of an applied electric field. In the present study, the location and distribution of the charged protein-rich particles along the surface of the electrode plate was investigated as a function of air flow rate (laminar *vs*. turbulent) and electric field strength. A turbulent air flow rate at a variety of electric field strengths resulted in the formation of protein-rich particles and starch granule agglomerates affecting the production of high-purity protein concentrates. Charging the flour particles at a laminar air flow rate followed by separation under a low electric field strength enabled the production of fine protein-rich fractions with considerably higher protein contents (38.4–46.5%) but lower protein separation efficiency. The combination of a laminar air flow rate and high electrode voltage slightly reduced the protein content of the fraction (39.4–42.9%), but significantly improved the protein separation efficiency to ~45%. To further improve the separation efficiency of the navy bean protein concentrate without compromising its protein content, a two-stage tribo-electrostatic separation approach was evaluated at a laminar air flow but different plate voltages. The combined protein-rich fraction produced by the two-stage approach had a protein content of ~38% accounting for 60% of the total protein that was significantly higher than that of the optimized single-stage tribo-electrostatic separation, facilitating the scale-up of this dry fractionation technique for pilot-plant applications.

1. Introduction

Wet fractionation processes are the most frequently used technologies for the production of relatively pure protein isolates (>90% protein) from plant sources. Conventional wet fractionation processes of plant proteins require organic solvents (e.g. petroleum ether/hexanes) at elevated temperatures for de-oiling seeds and grains, as well as water and chemicals for acid or alkaline solubilization and isoelectric precipitation [1,2]. More sustainable aqueous or enzyme-assisted aqueous extraction processes (AEP/EAEP) eliminate the use of organic solvents [3–6], but still require large amounts of water, alkali and/or concentrated acids for protein isolation.

Although wet fractionation technologies can produce isolates with a minimum of 90% protein, they are energy intensive due to the dehydration step required to convert the protein-enriched fractions into powdered form. Furthermore, the harsh processing conditions common to wet fractionation techniques are detrimental to the native functionality of the components as a result of protein denaturation and loss of protein solubility [7,8].

Dry fractionation is an alternative strategy to wet fractionation and can produce protein concentrates (~30–80% protein) with preserved native functionalities leading with lower energy requirements and no water [9,10]. Therefore, an efficient dry fractionation approach is very attractive since the resulting functional and/or nutritional protein-enriched fractions are promising ingredients in food formulation and processing as well as in value-added applications.

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Dry milling combined with air classification is the most commonly used dry fractionation technology for the production of protein-rich and starch-rich (protein-depleted) fractions from grain legumes [11–18]. Dry milling is initially applied for physical disentanglement of small protein fragments from large starch granules while air classification separates the milled legume flours into protein-enriched (light fine fraction) and starch-enriched particles (heavy coarse fraction) according to their aerodynamic properties, a combination of particle size and density [1,2,8,19]. However, dry milling and air classification cannot be successfully applied to a wide variety of legume grains and oilseeds since the separation principle in air classification depends greatly on particle size and density. Therefore, limited protein enrichment is obtained by air classification when disentangled particles differ little in size and/or density. Clearly, the development of an efficient dry protein enrichment approach with a different separation mechanism that could be easily expanded to a wide variety of plant resources is very attractive.

Attention to the development of tribo-electrostatic-based separation of agro and food materials is growing [20–25] while the principles of this separation technique have long been used by the pharmaceutical industry [26–28], mining [29–33] and the plastic industries [34,35]. Tribo-electrostatic separation methods have been industrially applied mainly by the mining sectors. Several tribo-electrostatic separation facilities and pilot plants have been set-up in the USA, Canada, UK, and Poland since 1995 processing over 1,000,000 tonnes of fly ash and minerals, confirming the scalability of the process [36].

Tribo-electrostatic separation is a two-step process comprised of tribo-electrification followed by the subsequent separation of the tribo-charged particles under the influence of an electric field. Tribo-electrification can occur by either electron or ion transfer mechanisms [37–39] through pneumatic conveying of particles along tubes as well as fluidized/vibrating beds where interparticle interactions on a substrate impart a surface charge to the particles [33,40,41].

We have validated a tribo-electrostatic separation approach for the fractionation of legume flour with the aim of producing protein- and carbohydrate-enriched fractions [9,42,43]. Protein particles charged easily upon physical contact with high work-function polytetrafluoroethylene (PTFE) tribo-charging medium due to the presence of ionizable functional groups, whereas carbohydrate particles did not charge effectively as they are characterized by low ionizability [43,44]. Subsequently, a vertical laboratory-scale tribo-electrostatic separation apparatus consisting of a fluidized bed, a PTFE tribo-charger, and a plate-type separation chamber was constructed to separate charged protein-rich particles from uncharged and/or weakly charged carbohydrate-rich particles [43]. The approach was optimized for the fractionation of navy bean (*Phaseolus vulgaris*) flour as a model system to evaluate the influence of several operating factors including air flow rate, tribo-charger tube length, and the plate voltage and angle on protein enrichment and separation efficiency [42]. It was found that plate angle over the range evaluated had no significant impact on the protein enrichment, whereas air flow rate, plate voltage, and tribo-charger tube length were found to have a significant impact on the protein enrichment and separation efficiency. The optimal values of air flow rate, tribo-charger length, plate voltage and plate angle were found to be 7 LPM, 240 cm, −6.5 kV and 20°, respectively [42]. The optimal approach resulted in a protein-rich fraction in its native state [9] containing ~38% protein from flour with an original content of ~25% protein [42].

The present study was carried out to determine how charged protein particles are distributed along the electrode plate under different operating conditions and how the accumulation of protein-enriched particles on the plate's surface could affect the degree of protein enrichment. The distribution of protein particles along the electrode plate was analyzed, and the effect of plate fouling on the protein enrichment and separation efficiency was evaluated. Multi-stage tribo-electrostatic separations were conducted using optimal operating conditions from the single-stage study to assess the promise of further protein enrichment and separation efficiency by taking this approach.

2. Materials and methods

2.1. Materials

Pin-milled navy bean (*Phaseolus vulgaris*) flour ($D_{4,3}$ = 70.2 ± 7.8 μm, $D_{3,2}$ = 35.1 ± 1.9 μm) containing 2.5 wt% oil (dry-basis), 25.4 wt% protein (dry-basis), 68.2 wt% carbohydrate (dry-basis), 3.9 wt% ash (dry-basis), and 6.6 wt% moisture was kindly provided by the Canadian International Grains Institute (CIGI, Winnipeg, MB, Canada). The flour was stored at −20 °C, and dried for 12 h at 70 °C prior to being fractionated. Potassium sulfate (99.0+%) and selenium oxychloride (97%) were from Sigma Aldrich (Oakville, ON, Canada) and used during the Kjeldahl digestion. Nessler reagent was from Ricca Chemical Company (Arlington, TX, USA) and employed in the direct nesslerization of the Kjeldahl digests.

2.2. Single-stage tribo-electrostatic separation

The tribo-electrostatic separation experiments were performed using a vertical laboratory-scale triboelectric separator (Fig. 1). The separator consisted of a fluidized bed flour reservoir, a PTFE tribo-charging tube, and a rectangular plate-type separation chamber. The layout of the separator designed by Advanced CERT Canada Inc. (Waterloo, ON, Canada) has been previously described [42,43].

For the single-stage tribo-electrostatic separation experiments, ~20 g of pin-milled navy bean flour was placed inside the fluidized bed. Dry air was used as a carrier for pneumatically transferring suspended particles into the PTFE tribo-charging tube (4.76 mm inside diameter × 250.0 cm length) and eventually into the separating chamber where a high negative voltage was applied to the copper-plate electrode so as to form an external electric field. The dry air flow through the tribo-charger tube was adjusted to 7 or 9 LPM. The separation studies were carried out at four different plate voltages ranging from −1 to −7.5 kV with a plate angle less than 20°. Following the single-stage electrostatic separation, four different fractions were collected from the chamber. Three fractions labelled as "PF1", "PF2" and "PF3" were collected from the bottom, middle, and top sections of the electrode plate, respectively (Fig. 1). The fourth fraction labelled "CF1" was collected in the bin located at the bottom of the chamber (Fig. 1). Earlier studies had shown that the navy bean protein particles are charged positively and attach to the plate while the carbohydrate-rich particles remain mostly uncharged, accumulating at the bottom of the chamber [43]. All fractions were analyzed for protein content. The yields (%) and percentages of total protein (%) were calculated based on the weight and protein content of each fraction using Eqs. (1) and (2), respectively.

$$\text{Yield } (\%) = (\text{mass (g) of each fraction}/\text{mass (g) of the original flour}) \times 100 \quad (1)$$

$$\text{Percentage of total protein } (\%) = (\text{protein (g) in each fraction}/\text{protein(g) in original flour}) \times 100 \quad (2)$$